

REMARKS

This is a full and complete response to the Office action dated November 1, 2006.

All comments and remarks of record are herein incorporated by reference. Applicants respectfully traverse these rejections and all comments made in the Office Action. Nevertheless, in an effort to expedite prosecution, Applicants provide the following remarks regarding the cited references.

DISPOSITION OF CLAIMS

Claims 1-17 are pending in the application. Claim 1 has been amended for clarification. Support can be found on page 12, ¶'s 47. Claim 17 was amended to add a period at the end of the claim. Claim 3 has been canceled. No new matter is added.

REJECTION UNDER 35 USC §112, FIRST PARAGRAPH

Claims 1-11 and 16-17 stand rejected under 35 USC §112, first paragraph as allegedly failing to comply with the written description requirement. Applicants respectfully traverse this rejection.

The Examiner alleges that there is no support for claim 1, and in particular no support for steps "obtaining a liquid suspension sample comprising different microorganisms...", "preparing a series of progressively dilute test samples..." and "utilizing an estimation model to determine the concentration of the viable..."

Applicants respectfully disagree. It is Applicants position that the Application as filed fully provides support for the amended claims.

Applicants respectfully assert that the recitation in claim 1 of "obtaining a liquid suspension sample comprising different microorganisms" is supported in the Application. As stated in the Application, "[t]he samples can be placed in a transport media, such as LBS broth." *See* Application, page 8, ¶29-30. Furthermore, it is recited in Example 2 of the Application, that "Liquid or solid media should be selected to be suitable for growth of the probiotic." *See* Application, page 8, ¶31. Furthermore, it is recited in the Application that "PCR can be performed on specific colonies growing on plates, or on

liquid culture samples.” *See* Application, page 11, ¶41. Therefore by placing the animal feed sample in the liquid media, a liquid suspension sample comprising different microorganisms is obtained.

Also, Applicants respectfully assert “preparing a series of progressively dilute test samples...” and “utilizing an estimation model to determine the concentration of the viable...” is supported in the Application. Applicants would like to note that preparing progressively dilute test samples is conducted in view of the “estimation model.” This can be seen by discussing an “estimation model” such as “most probable number” (MPN) in greater detail as indicated in the present application.

As discussed in the application, MPN is a “method for estimating low concentrations of organisms based on observation of serial dilutions.” *See* Application, page 12-13, ¶48 (emphasis added). MPN is known in the art, and two publications are cited in the Application on pages 12-13, ¶48 in order to show the state of the art with regard to MPN. These publications, Cochran, W. G. 1950, “Estimation of Bacterial Densities by Means of the ‘Most Probable Number’” (hereinafter “Cochran”), *Biometrics*, Vol 6:105-116, and James T. Peeler and Foster D. McClure; *Bacteriological Analytical Manual*, USFDA, 7th edition, 1992 (hereinafter “Peeler”) are provided herewith.

As indicated in Peeler, MPN is an estimate of the density of viable organisms in a sample. *See* Peeler, page 439, 3rd ¶. Furthermore, as indicated in **Cochran**, the “method consists in taking samples from [a] liquid, incubating each sample in a suitable culture medium, and observing whether any growth of the organism has taken place. *See* Cochran, page 105, 2nd ¶.

An illustrative example of the MPN method can consist of the following. A set of 3, 5 or 10 tubes, or other number containing a sample with a microorganism is taken. *See* Peeler, page 439, lines 20-21 of 3rd ¶. These sets of tubes are then diluted in series, usually by a ten fold dilution. The following is a simplified example of a set of 3 test tube series with 10 fold dilutions:

	Col. 1 +/-	Col. 2 +/-	Col. 3 +/-
Test tube series 1	V ₊	V ₊	V ₋
Test tube series 2	V ₊	V ₋	V ₋
Test tube series 3	V ₊	V ₋	V ₋
Dilution	10 ⁻¹	10 ⁻²	10 ⁻³
# of positives	3	3	1

In the above, as each row is more dilute than the previous, with each successive tube, it is more likely that the presence of the target microorganism will be too dilute to be detected. Therefore, in the case above, in Col. 3, it is more likely there will be a negative detection than in Col. 2. In the above example, if a microorganism is detectable, a "+" is put next to the test tube. If a microorganism is not detectable, a "-" is placed next to the tube. Furthermore, as can be seen in Col. 1, the tube in each series is positive, meaning the microorganisms are detectable at that dilution in each series. In Col. 2, there is 1 positive detection and 2 negative detections. However, in Col. 3, there are 3 negative detections.

Once having this information, one can use tables as provided on page 444 of Peeler for estimating the number of bacteria, shown in part below:

No. of positive tubes			MPN/g (ml) ^b
0.1	0.01	0.001	
0	0	0	<3
0	1	0	3+
1	0	0	4
1	0	1	7+
1	1	0	7
1	2	0	11+
2	0	0	9
2	0	1	14+
2	1	0	15
2	1	1	20+
2	2	0	21
3	0	0	23
3	0	1	39
3	1	0	43

For the simplified illustrative example above, one would look on such a chart in Peeler for [3, 1, 0] to obtain the MPN estimation of 43 MPN microorganisms per gram of original sample.

Thus the recitation of “preparing a series of progressively dilute test samples...” is shown and supported in the application, and especially wherein “serial dilutions” is recited directly in the application. *See* Application, page 12-13, ¶48. Also, as demonstrated above, recitation of “utilizing an estimation model to determine the concentration of the viable...” is supported in the application and well known as state of the art by those persons skilled in the art. *See* Application, pages 12-13, ¶48.

Furthermore, after “preparing a series of progressively dilute test samples” there is a step of “incubating the series of progressively dilute test samples” followed by “conducting a PCR analysis” as recited in claim 1.

Example 3 in the application shows serial dilution, incubating and then plating. *See* Application, page 9, ¶33; see also examples 4-6. However because of the high background of other microorganisms, the direct plate count method does not provide an accurate measure of the number of microorganisms. More specifically, if the background count of microorganisms is greater than 5 fold higher than the organisms of interest it is difficult if not impossible to detect or much less enumerate the organism of interest. Instead, the claimed method is used which includes a PCR analysis technique to provide clear evidence of the probiotic microorganism of interest. For the PCR step, in example 7 of the application it is recited that “the serial dilution cultures described in the previous Examples [as in example 3] can be used to amplify the ‘signal’ obtained from living organisms in the samples.” *See* application, page 12, ¶40. Subsequently, the application recites that “PCR can be performed on specific colonies growing on plates, or on liquid culture samples.” *See* application, page 12, ¶41.

It is further indicated in the application that PCR reactions using various known concentrations of standards can be used to quantify the concentration of probiotic in the culture. The application recites that “[t]his, combined with the degree of serial dilution, can be used to quantify the concentration of probiotic in the animal feed.” *See* application, page 12, ¶47. Thus, the above shows that the application provides support

for claim 1's recitation of "preparing a series of dilute test samples..." as well as the recitation of "incubating the series of progressively dilute test samples" and "conducting a PCR analysis."

As discussed above with respect to the MPN model, the PCR analysis is used to detect positive and negatives in the series of progressively dilute test samples. The incubation step assures that viable probiotic organisms will be detectable by the PCR analysis. Each of these steps is clearly discussed and shown in the application as filed.

Furthermore, the claimed method is conducted in Example 9 and compared to plate count method. This is shown as follows on page 13 of the application:

Sample	Plate Count	Most Probable Number
LA51 probiotic culture	$2.4 \times 10^{10} /g$	$2.0 - 2.4 \times 10^{10} /g$
Control feed	$1 \times 10^3 /g - 1 \times 10^7 /g$	0
Autolyzed (lab treated)	$5 \times 10^4 /g - 1.6 \times 10^5 /g$	$5 \times 10^4 /g - 1.6 \times 10^5 /g$
Treated feed	$5 \times 10^4 /g - 1.6 \times 10^7 /g$	$5 \times 10^4 /g - 1.6 \times 10^5 /g$

As can be seen above, enumeration of a LA51 probiotic culture by itself will have similar results with both a Plate count method and the claimed method. However, as can be seen with respect to "Control feed" above, the plate count method will show high amounts of organisms, while the MPN will show none. The control feed is feed that has not been treated with LA51, and therefore has only the background organisms. This shows the specificity of the claimed method. The Plate count method results in a large degree of overcounting due to the background levels of microorganisms, which are not LA51.

The Autolyzed (lab treated) feed is that which has been brought back to the lab, and sterilized, and treated with LA51, and therefore only LA51 will be present on such feed. Therefore, both the plate count and claimed method will measure a similar number of microorganisms. The treated feed has naturally occurring background and has been treated with LA51. As seen above, the plate count method results in a much higher number. This is because the plate count will show the number of all organisms, including

background, which are not LA51. However, the count according to the invention shows a more accurate count of the number of LA51 microorganisms. This example in the application provides further support for the method according to the present claims, and furthermore shows the advantageous results from its use.

In view of the foregoing, Applicants respectfully assert all steps are provided for in the Application, and therefore request that the above under 35 USC §112 rejection be withdrawn.

REJECTION UNDER 35 USC §112, SECOND PARAGRAPH

Claims 1-11 and 16-17 stand rejected under 35 USC §112, second paragraph, as being allegedly indefinite. Applicants respectfully traverse this rejection.

The Examiner alleged that “relative quantity” is indefinite. In making definiteness determination, the content of a particular application disclosure is taken into account. *See* MPEP §2173.01. As indicated in the application, the claimed invention can “quantify the concentration of probiotic in the animal feed.” *See* application, page 12, ¶47. Furthermore, the MPN is used to estimate the concentrations of organisms in a sample. *See* application, page 12-13, ¶48. Additionally, Applicants have clarified claim 1 to recite that the relative quantity of microorganism is in relation to a known amount of food product. Therefore, one of ordinary skill in the art can determine the scope of Applicants’ claimed invention in order to avoid infringement.

The Examiner also alleges that “substantial entirety” is unclear because it is not clearly defined in the specification and there is no art recognized definition for the phrase. In MPEP §2173.05(b) it is indicated that “substantially” is often used in conjunction with another term to describe a particular characteristic. Applicants respectfully assert that one of ordinary skill would easily understand the phrase because when a treated sample of feed is placed in liquid media, a substantial entirety of the viable microorganisms on such feed can be transferred and suspended to the liquid media resulting in a liquid suspension sample. *See* application, page 9-10, ¶’s 27-30. One of ordinary skill in the art would understand that an insignificant amount of microorganism may not be transferred.

The Examiner also alleged that the phrase “progressively dilute” is unclear. However, as discussed above serial dilutions is supported in the application on pages 12-13, ¶48 wherein it is recited that MPN “is a method for estimating low concentrations of organisms based on observation of serial dilutions.” Furthermore, as indicated above serial dilutions are made with a view to MPN. Therefore Applicants respectfully request that the above rejection be withdrawn.

Furthermore, the Examiner alleged that the phrase “estimation model” is unclear; Applicants respectfully traverse. Applicants respectfully note that the application recites that MPN “is a method for estimating low concentrations of organisms based on observation of serial dilutions.” See Application, page 12-13, ¶48. Furthermore, it is indicated in Peeler that MPN is an estimate of the density of viable organism in a sample. See **Peeler**, page 439, 3rd ¶. MPN is also based on a mathematical probability model. See **Cochran**, page 106-109. Therefore, one of ordinary skill in the art would understand the phrase “estimation model” and be apprised of the claimed scope.

In view of the foregoing, Applicants respectfully request that the above under 35 USC §112 rejection be withdrawn.

REJECTION UNDER 35 USC §102(b)

Claims 1-2, 4, 7-8, 10, and 16-17 stand rejected under 35 USC §102(b) as being anticipated by **Begum et al.**, Molecular and Cellular Probes 1995, (hereinafter “**Begum**”). Applicants respectfully traverse this rejection.

The Examiner alleges that **Begum** is directed to the detection of Shiga-like toxin producing Escherichia coli (SLTEC) using PCR and therefore anticipates the present claims. See **Begum**, abstract. The Examiner further alleges that **Begum** discloses diluting the ground beef samples as well as enrichment before PCR analysis. The Examiner additionally contends that Table 3 in **Begum** discloses a list of strains, sources, the dilution, and how much was detected. In light of the above, the Examiner concludes that the instant claims are anticipated. Applicants respectfully disagree.

It is Applicants position that **Begum** does not disclose the present claims. Applicants respectfully assert that **Begum** merely detects the presence or absence of

SLTEC, and does not use the detection of SLTEC along with PCR to determine the concentration of the microorganism in the ground beef sample. Furthermore, Applicants respectfully assert that Table 3 on page 261 of **Begum** does not show “how much was detected” as alleged by the Examiner, but merely shows the detection limits. No determination of concentration or quantity of SLTEC in the original sample is disclosed or suggested by **Begum**.

As stated in **Begum**, the purpose of the study disclosed therein is for the direct identification of SLTEC in ground beef samples without prior isolation of the strain or its DNA. *See* **Begum**, pg 262, 2nd column, Discussion. Applicants respectfully note to the Examiner that the concentrations discussed in **Begum** were known in advance. **Begum** does not disclose or suggest any method for determining the concentrations, without knowing such concentrations beforehand.

For example, the beef samples were inoculated with known quantities of SLTEC. *See* **Begum**, pg 263, 1st column, 2nd ¶. Dilutions were made only to reduce the concentration of components which may inhibit the PCR reaction. *See* **Begum**, pg 261, 2nd column. Furthermore, enrichment was used for only those strains having poor detection limits. *See* **Begum**, pg 263, 1st column, 2nd full ¶. Thus, the concentrations were adjusted in **Begum** so that the detection limits would not be too low for detection by PCR analysis. *See* **Begum**, pg 263, 1st column, 2nd ¶; also Table 3. After PCR, no further estimation model is disclosed or suggested to arrive at the original concentration.

Applicants respectfully note that without knowing the concentration of SLTEC beforehand, the enrichment step would cause unknown growth of the particular organism. Therefore, if concentration was then found after such enrichment step, this would provide no quantitative information of the original sample, and in fact would obscure the concentration of the original sample.

Therefore applicants respectfully assert that **Begum** does not disclose the presently claimed invention. As indicated above, **Begum** does not disclose or suggest utilizing an estimation model to determine concentration based on the results of a PCR analysis. Thus, Applicants respectfully request that the 35 USC §102(b) rejection be withdrawn.

REJECTION UNDER 35 USC §103(a) IN VIEW OF THOMAS

Claim 3 stands rejected under 35 USC §103(a) as being allegedly unpatentable over **Begum** in view of **Thomas**, (Applied and Environmental Microbiology 1991). Applicants respectfully traverse this rejection.

Applicants have canceled claim 3, and therefore the above mentioned rejection is no longer applicable. Favorable action is solicited.

REJECTION UNDER 35 USC §103(a) IN VIEW OF PAHUSKI

Claims 5-6 stand rejected under 35 USC §103(a) as being allegedly unpatentable over **Begum** in view of **Pahuski et al.**, US Patent No. 5,587,286 (hereinafter “**Pahuski**”). Applicants respectfully traverse this rejection.

Applicants respectfully re-assert the remarks made above with respect to **Begum**. Furthermore, Applicants respectfully submit **Pahuski** does not add anything to **Begum** which would remedy the failure to disclose or suggest the presently claimed invention. Even in view of **Pahuski**, **Begum** does not disclose or suggest utilizing an estimation model to determine concentration based on the results of a PCR analysis. Thus, Applicants respectfully request that the 35 USC §103(a) rejection be withdrawn

REJECTION UNDER 35 USC §103(a) IN VIEW OF LUCCHINI

Claim 9 stands rejected under 35 USC §103(a) as being unpatentable over **Begum** in view of **Lucchini et al.**, Federation of European Microbiological Societies, (hereinafter “**Lucchini**”). Applicants respectfully traverse this rejection.

Applicants respectfully re-assert the remarks made above with respect to **Begum**. Furthermore, Applicants respectfully submit **Lucchini** does not add anything to **Begum** which would remedy the failure to disclose or suggest the presently claimed invention. Even in view of **Lucchini**, **Begum** does not disclose or suggest utilizing an estimation model to determine concentration based on the results of a PCR analysis. Thus, Applicants respectfully request that the 35 USC §103(a) rejection be withdrawn.

REJECTION UNDER 35 USC §103(a) IN VIEW OF DESROSIER

Claim 11 stands rejected under 35 USC §103(a) as being unpatentable over **Begum** in view of **DesRosier et al.**, US 4,868,110, (hereinafter “**DesRosier**”). Applicants respectfully traverse this rejection.

The Examiner alleges that **DesRosier** teaches that the MPN test is frequently used for estimating the amount of bacteria in food and water samples. The Examiner further alleges that it would have been obvious to one of ordinary skill in the art to have modified the method of **Begum** so as to quantify the amount of bacteria in the sample using the MPN test as suggested by **DesRosier** for the benefit of using a procedure that utilizes liquid growth. Applicants respectfully disagree.

Applicants respectfully assert that the Examiner is engaged in impermissible hindsight reconstruction, using the Applicants’ specification as a roadmap. *See* MPEP §2145 X.A. There is no motivation to combine the references and furthermore, even if done, would not disclose all of the limitations of claim 16.

Begum is directed to merely the detection of the presence or absence of a particular microorganism, SLTEC, on a sample of beef by use of PCR analysis. *See* **Begum**, abstract. **Begum** further discloses the lower detection limits at which such detections are still possible. *See* **Begum**, Table 4. As discussed earlier, **Begum** does not disclose or suggest any enumeration methodology for determining concentration.

DesRosier discloses the use of MPN for estimating the number of viable organisms in a sample. *See* **DesRosier**, Col 2, line 16 to Col. 3, line 2. **DesRosier** further discloses that the release of a certain gas produced by the target microorganism can be used to indicate the presence of that target microorganism. *See* **DesRosier**, Col 2, line 64 to Col. 3, line 20. That information can then be used for enumeration by use of MPN. *See* **DesRosier**, Col 2, line 64 to Col. 4, line 56. **DesRosier** then discloses a gas collection and detection device for estimation by the MPN procedure. *See* **DesRosier**, Col 4, line 59 to Col. 6, line 26.

Applicants respectfully assert that because **Begum** is merely directed to detecting the presence or absence of SLTEC, one of ordinary skill in the art would have no

motivation to use the MPN methodology as disclosed in **DesRosier** for the process disclosed in **Begum**.

Furthermore the instant claim 16 recites that the MPN method is used to determine the concentration of viable microorganisms of interest based on results of the PCR analysis. Even if **Begum** were modified in view of **DesRosier**, it does not disclose all the limitations of claim 16, as neither **Begum** nor **DesRosier** teach or suggest that MPN concentration method is based on PCR analysis. **DesRosier** discloses the detection of gas released by the target microorganism for use in the MPN method, and not PCR.

Furthermore, while **Begum** mentions dilution, it does not teach the serial dilutions should be made in such manner that would be useful for the MPN method.

Additionally, **DesRosier** discloses that MPN procedure is useful with regard to low microbial density, citing a limit of 10 organisms/gm or less. On the other hand, the detection limits disclosed in **Begum** are from 30 to 220,000 ml⁻¹, which is outside the range disclosed by **DesRosier**, and so one of ordinary skill in the art would not be motivated to use the PCR method disclosed in **Begum** for MPN. In fact, because the detection limits in **Begum** are higher than 10 organisms/gm, **DesRosier** in fact teaches away from their combination and teaches away from use the PCR analysis as disclosed in **Begum**.

At least for all these reasons, no prima facie case of obviousness can be established. Furthermore, Applicants respectfully submit that virtually all inventions are combinations of old elements, however, the current claimed invention must be considered “as a whole”. See *Princeton Biochemicals Inc. v. Beckman Coulter*, 411 F.2d 1332, 1337, 75 USPQ.2d 1051 (Fed. Cir. 2005); 35 USC §103(a). Without this requirement, examination of an application “might break the invention into its component parts (A+B+C), then find a prior art reference containing A, another B, and another containing C, and on that basis alone declare the invention obvious.” See *Richard Ruiz v. Chance*, 357 F.3d 1270, 1275 69 USPQ.2d 1686 (Fed. Cir. 2004). This is a form of hindsight reasoning. See *id.*

While PCR on its own may be known, and on the other hand, MPN may be known, the claim as a whole is not obvious in view of the cited references. Therefore

Applicants respectfully assert that no prima facie case of obviousness is shown and therefore request the above 35 USC §103 rejection be withdrawn.

NON-STATUTORY DOUBLE PATENTING REJECTION

Claims 1-11 and 16-17 stand provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1, 7, and 9-16 of copending Application No. 10/711,155. Pending client documentation, a terminal disclaimer will be filed with respect to the above mentioned claims.

The undersigned representative requests any extension of time that may be deemed necessary to further the prosecution of this application.


Please charge any shortage in fees due in connection with the filing of this paper, including Extension of Time fees, to Deposit Account No. 14.1437. Please credit any excess fees to such deposit account.

In order to facilitate the resolution of any issues or questions presented by this paper, the Examiner is invited to directly contact the undersigned by phone to further the discussion.

Conclusion

Having addressed all issues set out in the Office action, Applicants respectfully submit that the claims are in condition for allowance and respectfully request that the claims be allowed.

Respectfully submitted,
NOVAK DRUCE & QUIGG, LLP


Jason W. Bryan
Reg. No. 51,505

1000 Louisiana Ave
53rd floor
Houston, Texas 77002
T: 713-571-3400
F: 713-456-2836